REMARKS

By the above amendments, the specification has been amended to make reference to corresponding sequence identification numbers in the Sequence Listing.

A Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures indicating the need to provide sequence numbers for sequences in Figure 5 and otherwise where appropriate in the specification was included in the Office Action. This is believed to have been accomplished by the amendments to the specification and it is further noted that the originally submitted Sequence Listing was complete and included all of the sequences in the specification and the drawings. That Sequence Listing was submitted with a paper dated October 27, 2003 in this application. Thus, it is believed that the requirements of the present Notice have been met and it should not be necessary to submit a duplicate Sequence Listing, however, applicants are ready to do so if required. A copy of the Notice is being returned with this paper.

The amendments to the claims include amendments to claims 53, 57, 71 and 81-86. Claims 54-56, 58-70, 76-77 and 89-106 have been canceled without prejudice or disclaimer of any subject matter therein. No claim has been allowed.

In the present Action, the Examiner has rejected claims 53, 55-72, 74-76 and 78-88 under 35 USC § 112, second paragraph, and lists a variety of formal objections. It is believed that the amendments to the claims have addressed and resolved all of these concerns. Thus, claim 53 has been amended and made more definite and clearly indicates that the "chromatin inactivation portion" of the polypeptide is a separate part of the polypeptide from the "nucleic acid binding portion". Therefore, the nucleic acid binding portion does not bind to the "chromatin inactivation portion".

Other spelling and grammatical corrections have been made. Claim 57 is now clearly a sentence and in claim 81, "gene" has been corrected to "genes". Claim 82, 83 and 85 have been put in proper method form.

It is noted that several rejections have been made with regard to 35 USC § 112, first paragraph. In the first instance, claims 53, 55-72, 74-76 and 78-88 have been rejected as failing to comply with the written description requirement. Applicants respectfully traverse this rejection.

In this regard, at the outset it should be noted that the amended claims limit the DNA-binding portion to a "DNA binding part of a nuclear receptor DNA binding protein" and also limit the chromatin inactivating portion to "all or a N-CoR and SMRT-

binding part of PLZF". In this manner, the polypeptide now requires both a DNA binding part of a nuclear receptor DNA binding portion and a chromatin inactivating portion which is all or N-CoR or SMRT-binding part of PLZF. Therefore, the amended claims include defined structural features for both the DNA binding portion and the chromatin inactivating portion. Supporting examples of such polynucleotides and polypeptides can be found in the specification at pages 44-50. Applicants respectfully request that this rejection be withdrawn.

On pages 6 and 7 of the Official Action, claims 53, 55-72, 74-76 and 78-88 have been rejected under 35 USC § 112, first paragraph, because the specification is deemed to be not enabling for a person skilled in the art to which it pertains, or which it is most nearly connected, to make and/or use the invention commensurate with the scope of the claims. Applicants respectfully traverse this rejection and believe that circumstances including evidence available as of the filing date of the application shows that claims of the present scope are adequately enabled. In this regard, certain supporting evidence has been generated and is submitted herewith in the form of Annex I for entry and consideration by the Examiner in support of adequate enablement.

The data presented in Annex I, and based on Example 1, beginning at page 44 of the specification, demonstrates that a PLZF-ER polypeptide can suppress the expression of *endogenous* oestrogen-responsive genes in human cell lines. For example, Section 2 and Figures 2 and 3 of Annex I demonstrate that there is a reduction in the expression of *endogenous* oestrogen-responsive genes in cell lines which express PLZF-ER. It is believed that this adequately demonstrates that one skilled in the art based on the content of the original specification can make and use the invention commensurate in scope with the present claims.

It should be noted that Section 112 requires that the patent specification enable one skilled in the art to make and use the full scope of the claimed invention without "undue experimentation". It is not believed that performing a few confirming experiments, as is the case here, amounts to "undue experimentation". Furthermore, the enablement requirement is met if the description enables "any mode of making and using the invention". JOHNS HOPKINS UNIVERSITY et al v. CELLPRO, INC., 152 F.3d 1342, 1361 (Fed.Cir. 1998) See, also, for example, INVITROGEN CORPORATION v. CLONTECH LABORATORIES, INC., 429 F.3d 1052; (Fed.Cir. 2005); and CAPON et al v. ESHHAR et al v. Jon Dudas, 418 F.3d 1349; (Fed.Cir. 2005).

As the cases indicate, the level of those skilled in the art is recognized as being quite high and the need for a great deal of detail in order to meet the enablement requirement is reduced accordingly. In view of the amended claims, supporting evidence and defined requirements consistently reflected by recent court decisions, applicants strongly believe that the enablement requirement has been met.

Finally, at pages 7-9 of the Official Action, the Examiner has raised the issue of the predictability (or unpredictability) of the art as related to the amount of direction or guidance presented in the specification in the presence or absence of working examples. These issues have been addressed, at least in part, in earlier remarks and applicants believe that based on the state-of-the art at the filing date of the present invention adequate predictability existed in the targeting and delivery of polypeptides to cells. Applicants believe that their position is on sound ground, however, they offer certain additional evidence in support of adequate predictability available to those skilled in the art.

At the time of filing this application, liposome transfection was a well-known and routine method for the intra-cellular delivery of polypeptides and proteins to eukaryotic cells. Lipofectin (the first commercial reagent for this

method) was launched in the late 1980's. Early examples of liposome transfection of polypeptides and proteins to eukaryotic cells include the delivery of prostatic acid phosphatase protein into human prostate carcinoma cells in 1993, and the cytoplasmic delivery of polypeptides in 1990. In support of this position, the applicants offer several exemplary publications which they enclose and have also made the subject of a Supplemental Information Disclosure Statement which accompanies this Amendment to make these references officially of record in this application.

In regard to the above, see Lin et al Biochem Biophys Res Commun. 1993 Apr 30;192(2):413-9; and Chu et al Pharm Res. 1990 Aug;7(8):824-34.

Furthermore, applicants are convinced that the targeting and delivery of polypeptides to target cells in accordance with the invention involves peptide sequence delivery technologies that were established and could be used successfully at the time of filing of the present application. No particular expertise was required, the technology being well within the grasp of those skilled in the art.

Successful application of the technology is further shown in the literature by Lindgren et al, Trends Pharmacol Sci. 2000

Mar;21(3):99-103 and Hawiger, Curr Opin Chem Biol. 1999 Feb;3(1):89-94.

For the above and other reasons, applicants submit that achieving sufficiently predictable intracellular delivery of polypeptides involves nothing more than what was standard laboratory process at the time of filing the present application.

Accordingly, the Examiner is respectfully requested to reconsider her position, withdraw the rejections under 35 USC § 112 and allow the claims. Entry of Annex I is also respectfully requested.

Respectfully submitted,
NIKOLAI & MERSEREAU, P.A.

C. G. Mersereau
Attorney Registration No. 26,205
Attorney for Applicants
900 Second Avenue South
Suite 820 International Centre
Minneapolis, MN 55402
Telephone: (612) 339-7461

CERTIFICATE OF MAILING

I hereby certify that the foregoing Amendment in response to the Official Action mailed August 9, 2005, a Transmittal Letter, a Supplemental Information Disclosure Statement, Petition for a three-month extension of time, together with a check in the amount of $1,200.00, in application Serial No. 10/019,520, filed June 10, 2002, of Lakjaya Buluwela et al, entitled "CONTROL OF GENE EXPRESSION", and a transmittal letter are being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, postage prepaid, on February 9, 2006.

Barbara L Davis

Barbara L. Davis
On behalf of C. G. Mersereau

Date of Signature: February 9, 2006

Annex 1: Further supporting data

Section 1: Establishment of MCF-7 cell lines expressing PLZF-ER in a tetracycline-inducible manner

PLZF-ER (as discussed in Example 1 of the application) was cloned into the TET-off plasmid pREVTRE (Clontech), which also confers resistance to hygromycin, and the recombinant plasmid stably transfected into the MCF7 TET-off (TO) cell line (Clontech). MCF7 is a human breast cancer cell line. Growth of MCF7 is dependent upon the presence of oestrogen in the growth media.

In the presence of doxytetracycline (TET65; 100 μg/ml) in the growth medium, the transformed MCF7 cell lines constitutively expresses the TET repressor and represses expression of genes held in the pREVTRE plasmid. Three different populations of cells were selected for further investigation. MCF7 Pool is a pool of cells that survived hygromycin selection, whilst JP13 and JP23 are two representative and independent clones from that pool.

MCF-7-TO (the parental cell line), MCF7 Pool, JP13 and JP23 cells were transiently transfected with an estrogen-regulated reporter gene, chloramphenicol acetyl transferase (CAT), containing an ERE (ERE-G-CAT). Following removal of TET and/or the addition of 17ß-estradiol (E2; 10 nM) reporter gene activity was assessed and the data is presented in Figure 1 panel A. Reporter gene activity was similar in MCF7-TO in the absence or presence of TET and in the MCF7 pool, JP13 and JP23 cell lines in the presence of TET. In the absence of TET, however, reporter gene activity was significantly reduced in the MCF7 pool, JP13 and JP23 cell lines. The results represent at least 3 independent experiments. In each experiment ER activity in the presence of E2 was taken as 100%. All other activities are shown relative to this.

To confirm that the changes in CAT activity were associated with PLZF-ER protein levels, immunoblotting of whole cell extracts prepared from MCF-7-TO, MCF7 pool, JP13 and JP23 cells grown in the presence or absence of TET was performed using an anti-PLZF antibody. The results are presented in Figure 1, panel B.

The data shows that TET+ oestrogen (E2)+ cells have CAT reporter gene activity. This is due to the TET repressing the expression of PLZF-ER from the pREVTRE plasmid and E2 inducing transcription of the oestrogen-responsive CAT reporter gene.

When TET is omitted from the growth media then PLZF-ER is transcribed and, in the case of E2+ cells, the PLZF-ER protein is activated and blocks transcription of the CAT reporter gene. In the absence of E2 no CAT activity is recorded as oestrogen is required to activate the reporter gene and to activate PLZF-ER.

Hence MCF-7 cell lines JP13 and JP23 express PLZF-ER in a tetracycline inducible manner. Also, expression of PLZF-ER is associated with a decrease in CAT reporter gene activity in an E2 – dependent manner.

Section 2: Expression of an endogenous oestrogen-responsive gene in PLZF-ER cell lines

In Section 1 we established that the tetracycline-inducible PLZF-ER construct could down-regulate the activity of an oestrogen-responsive reporter gene.

We now describe a series of experiments that demonstrate that the PFZF-EF construct can regulate the expression of progesterone receptor (PR), an *endogenous* oestrogen - responsive gene. The gene expression levels were analysed using RT-PCT, a technique well known to those skilled in the art.

MCF-7 cells (MCF-7 TO), MCF-7 Pool cell and the cell lines JP13 and JP23 (described in section 1) were grown in the presence or absence of TET (100 μg/ml), with 17ß-estradiol (10 nM) present throughout the course of the experiment. Total RNA was extracted from the cells using Qiagen RNAeasy kits (Qiagen, UK). 2 μg of total RNA was used to make randomly primed cDNA. This cDNA was then used as a template for a PCR reaction using primers for progesterone receptor (PR) and GAPDH. The PR and GAPDH primers used were:

		Accession No.	Base Pairs
PR primers:			
PR-F2	AAATCATTGCCAGGTTTTCG	AF016381	2375-2395
PR-R2	TGCCACATGGTAAGGCATAA	AF016381	2584-2564
GAPDH Primers:			
GAPDH-F	CCACCCATGGCAAATTCCATGGCA	NM002046	229-252
GAPDH-R	TCTAGACGGCAGGTCAGGTCCAC	NM002046	802-824

The PCR conditions used were: 27 cycles, annealing temp. 50°C for the PR RT-PCR product of 238bp; 30 cycles, annealing temp. 55°C for the house-keeping (control) gene GAPDH RT-PCR product of 595bp.

The data from this experiment is shown in Figure 2. It can be clearly seen that when TET is omitted from the cell growth conditions (-) there is a reduction in the expression of the PR gene. Hence in cell lines where PLZF-ER is expressed (see Figure 1 panel B for PLZF-ER expression in TET- cell lines) there is a reduction in the expression of an endogenous oestrogen – responsive gene.

In order to established whether there was a reduction in progesterone receptor (PR) protein levels in TET- cells, we conducted an immunohistological staining for PR protein in these cells, a technique well known to those skilled in the art.

MCF-7 Tet Off cell line and PLZF-ERα stably transduced MCF-7 cell lines JP13 and JP23 (described in Section 1) were grown in complete medium in the presence of 10^{-8} M E2 and Doxytetracycline (1μg /ml). The cells were harvested by trypsinisation and subsequently spun onto the surface of glass microscope slides ("cytospun"), air dried, fixed and immunostained with a mouse monoclonal antibody against human PR (Biogenics), with staining visualised using a secondary antibody (Vector laboratories) conjugated to Horseradish peroxidase (HRP). Positive staining is brown in colour and localised to the cell nucleus. Immunostained cells were counter stained with Haematoxylin - Eosin (H&E), which stains the cell nuclei blue and cytoplasm red. The results are shown in Figure 3. Cells are shown at a magnification of x40.

As seen in panel (A), strong nuclear expression of PR is seen in MCF7-Tet Off cells. Also detectable in these cells is weaker, cytoplasmic staining, which is known to be a background staining effect. In comparison, nuclear expression of PR is greatly reduced in the PLZF-ERα stable lines JP13 (B) and JP23 (C). These lines, however, show some weak cytoplasmic, background, staining seen in the parental line.

Hence expression of the PLZF-ER construct is associated with a reduction in the protein levels of PR, an *endogenous* oestrogen-responsive gene.